Degradation of macromolecules by microfungi isolated from different podzolic soil horizons

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The abilities of 60 species of soil microfungi to decompose protein, xylan, cellulose, and chitin were tested with an agar diffusion technique. Proteolytic capacity was shown by 51 species; 35 were xylanolytic, 31 were cellulolytic, and 23 were chitinolytic. The importance of the physiological capacities of soil fungi in determining the communities in different soil horizons is discussed.

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La capacité de 60 espèces de microchampignons du sol de décomposer les protéines, le xylane, la cellulose et la chitine a été évaluée à l'aide d'une technique de diffusion sur gélose. Cinquante-et-une espèces se sont avérées être protéolytiques; 35 étaient xylanolytiques, 31 étaient cellulolytiques et 23, chitinolytiques. Les auteurs discutent l'importance des capacités physiologiques des champignons du sol pour la détermination des communautés dans divers horizons du sol.

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Introduction

Microfungal species composition has been studied, more or less intensively, in many soils throughout the world. These investigations now enable us to make reasonably good predictions on which fungi may be found in different soils. Knowledge of the degrading activities of these soil fungi is, however, considerably less. Undoubtedly they play a major role in the decomposition processes and, thus, their physiological properties, including degradative ability, are of great ecological interest.

Many different methods to test the degrading ability of fungi have been used and the quantitative results from different investigations are therefore difficult to compare. Information on physiological capacities of different fungi are scattered throughout the literature, but more extensive data are given by Borut (1960), Loub (1960), Domsch and Gams (1969), Nilsson (1974), and Flanagan and Scarborough (1974), among others. An excellent review concerning fungi from arable soils is given by Domsch and Gams (1970). Still, the physiological properties of many fungal species common in soil are unknown. This was realised during a study of the microfungal flora of some Swedish coniferous forest soils (Söderström 1975; Söderström and Bååth 1978). These investigations also showed that distinct different fungal communities exist in the different soil horizons of podzols. The reason for this is obscure, but physiological properties of the different fungal species could be one important factor regulating the vertical distribution of the species.

The ability of microfungi from coniferous forest soils to decompose common and important macromolecules was therefore investigated. The macromolecules studied were protein, xylan (hemicellulose), cellulose, and chitin. Using some of the data on the frequency of isolation of microfungi from Söderström and Bååth (1978), the degrading potential of the microfungal communities in different soil horizons is discussed.

However, in any study of this kind one must bear in mind that the fungi isolated on the laboratory media comprise only part of the fungal soil flora. The Basidiomycetes, for instance, are seldom isolated (e.g., Söderström and Bååth 1978). Furthermore, the fact that a fungus can decompose a certain substance in pure culture does not necessarily imply that it exhibits the same function in its natural habitat and *vice versa*. Physical, chemical, and biological factors could be important in modifying the physiological ability of a species.

Materials and methods

All the tested fungi were isolated from coniferous forest soils by a soil washing technique. They were kept as pure cultures on slants of malt extract agar (2% malt extract (Oxoid), 1.5% agar (Difco)) at 4°C until the tests were performed. Many species were represented by more than one isolate, obtained from different soil horizons.

The ability of the fungi to decompose macromolecules was assessed by an agar diffusion technique according to Rautela and Cowling (1966). The following substances were used: pro-

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tein (skim milk powder (Difco)) 1.5% w/w, xylan (Sigma) 0.25% w/w, cellulose (Munktell's cellulose powder No. 400) 0.28% w/w, chitin (Fluka) 0.30% w/w. Xylan was ground in a mortar for 5 min before use. Cellulose powder was swollen in 85% phosphoric acid at 0°C, regenerated and washed in distilled water, and finally neutralized with NaOH. Chitin was dissolved in concentrated HCl at 4°C, filtered through glasswool, precipitated in 50% ethanol, and finally washed in distilled water.

The test substances were combined with a basal medium composed as follows: 2 g $NaNO_3$, 1 g $(NH_4)_2SO_4$, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.01 g $CuSO_4 \cdot 5H_2O$, 0.005 g $MnSO_4 \cdot 4H_2O$, 0.001 g $ZnSO_4 \cdot 7H_2O$, 0.05 g $CaCl_2$, 13 g agar (Difco), and 1000 mL distilled water. The pH of the medium was 6.4 for protein, 5.3 for xylan, 6.0 for cellulose, and 5.4 for chitin. The optimal pH was chosen after running a test series at different pH values with a number of different fungi. In preliminary tests, small quantities of glucose were incorporated in the basal medium. As this resulted in no improvement in degrading capacity of the fungi, glucose was omitted from the main experiment.

Sterile, uniformly opaque columns (2–4 cm in height) of the test substances in the basal medium were prepared in test tubes (13 mm in diameter). They were inoculated with agar discs (9 mm in diameter) with mycelium taken from the edge of actively growing colonies on carboxymethylcellulose agar containing 0.5% yeast extract (Gams 1960).

The test tubes were incubated at 23°C in plastic bags to avoid excess drying of the columns. The depth of clearing in the test tubes with protein or xylan was measured after 12 days, whereas the tubes with cellulose and chitin were measured after 49 days. A fungus producing a clear zone of more than 10 mm was classified as having good degrading capacity. After 70 days all tubes were checked again for signs of clearing and the purity of the organisms was checked visually. Each isolate was tested on all four substrates and all tests were performed in duplicate.

Results

Table 1 shows the ability of 60 species of soil microfungi to decompose protein, xylan, cellulose, and chitin. When more than one isolate was tested, very small differences were noted between the isolates and thus a mean value was calculated. Of the total number of species tested, 51 were proteolytic, 35 were xylanolytic, 31 were cellulolytic, and 23 were chitinolytic.

Six species were able to clear all the test media, namely *Mortierella nana*, *M. ramanniana*, *M. vinacea* I, *Trichoderma hamatum*, *T. viride*, and *Verticillium bulbillosum*. For three species no degrading capacity was detected, namely *Aureobasidium* sp. 1, *Septonema chaetospira* var. *pini*, and *Thysanophora penicillioides*.

The highest xylanase activity, i.e., producing the deepest clear zones in the tubes, was shown by *Penicillium daleae*, *Trichoderma hamatum*, and *T. viride*. *Botrytis* sp. and the three species of *Trichoderma* together with *P. daleae* showed the best cellulose degrading ability, whereas *Mortierella macrocystis*, *Paecilomyces farinosus*, *Verticillium bulbillosum*, and *V. lecanii* were the best chitin decomposers.

Some of the species listed in Table 1 have been studied previously, but some of the present results concerning degrading ability do not appear to have been reported.

Discussion

Members of the genus *Mortierella* and *Penicillium* were the most frequently isolated microfungi in Swedish coniferous forest soils (Söderström 1975; Söderström and Bååth 1978) and they appear to be very common in most temperate soils. The chitinolytic abilities among species of *Mortierella* have frequently been reported and in the present study only 1 out of 14 species tested lacked this ability.

In contrast to the genus *Mortierella*, no species of *Penicillium* was able to attack chitin in this study. Instead most of them were xylanolytic and cellulolytic, which in turn was in contrast to most of the *Mortierella* species. These facts may indicate a generic niche separation based on nutrition.

There were no great differences in degrading capacity between different isolates of the same species, but such differences were indeed detected between different species within a genus. Domsch and Gams (1969) emphasized the importance of determining the organisms to species level in soil ecological studies, and our results support their conclusion.

Apart from contributing to the knowledge of degrading capacities of common soil microfungi, the study was also made as an attempt to find some explanation for the different horizontal preferences of the fungi reported earlier (Söderström 1975; Söderström and Bååth 1978). Plant litter reaching the soil surface by litterfall undergoes considerable chemical changes during incorporation into the different soil horizons. One would therefore expect the fungal communities in the different soil layers to exhibit different physiological capacities. However, when the relative degrading capacities of the fungal communities of three coniferous forest soils were compared (Figs. 1A–1C), no general tendencies were found. The relative degrading capacity was here calculated as the percentage of isolates with any degrading capacity of the total number of isolates, using the data of vertical distribution of the species given by Söderström and Bååth (1978). Only species given in Table 1 were included, but they represented a major part of the total number of isolates. Our results are in accordance with the findings of Flanagan and Scarborough (1974), that

TABLE 1. The ability of 60 species of soil fungi to degrade protein (Pr), xylan (Xy), cellulose (Ce), and chitin (Ch); ○, no decomposition; ◐, slight decomposition; ●, good decomposition

Fungal species	Test substance				Horizon with highest frequency of isolation
	Pr	Xy	Ce	Ch	
Absidia cylindrospora Hagem	●	○	○	◐	A_1/A_2
Acremonium cf. *griseoviride* (Onions & Barron) W.Gams II	◐	○	○	○	A_{01}
Aureobasidium pullulans (de Bary) Arnaud	◐	○	○	○	A_{00}
Aureobasidium sp. 1	○	○	○	○	A_{00}, A_{01}
Beauveria bassiana (Balsamo) Vuill.	●	○	○	◐	A_{00}
Botrytis sp.	●	●	●	○	A_{00}
Candida sp. 1	◐	●	◐	○	A_{01}
Ceuthospora pinastri (Fr.) Höhnel	◐	●	◐	○	A_{00}
Chrysosporium pannorum (Link) Hughes	◐	●	◐	○	A_1/A_2
Cladosporium cladosporioides (Fres.) de Vries	●	●	●	○	A_{00}
C. herbarum (Pers.) Link ex Fr.	●	◐	◐	○	A_{00}
Cylindrium sp. 1	◐	◐	◐	○	B
Cylindrocarpon destructans (Zins.) Scholten	●	●	◐	○	A_{00}, A_{02}
Hormonema sp.	○	◐	○	○	A_{00}
Humicola fuscoatra Traaen	○	●	◐	○	A_{02}, A_2
Mariannaea elegans (Corda) Samson var. *elegans*	●	●	◐	○	A_{00}
Mortierella alpina Peyron	●	○	○	◐	B
M. echinula Linnemann	●	○	○	◐	A_{01}
M. humilis Linnemann	●	○	○	◐	A_{02}
M. isabellina Oudem.	●	○	○	◐	A_{01}, A_{02}
M. jenkinii (Smith) Naumov	◐	○	○	◐	A_{01}
M. macrocystis W.Gams	◐	○	○	◐	A_2
M. minutissima van Tieghem	●	○	○	◐	A_1/A_2
M. nana Linnemann	◐	◐	◐	◐	B
M. parvispora Linnemann	◐	○	○	◐	A_{01}, A_{02}
M. ramanniana (Möller) Linnemann	●	●	●	◐	A_{00}, A_{01}
M. verticillata Linnemann	●	○	○	◐	A_{01}
M. vinacea Dixon-Stewart I	◐	◐	◐	◐	A_1/A_2
M. vinacea II	◐	○	○	○	B
Mortierella sp. 1	●	○	○	◐	(B)
Mucor hiemalis Wehmar	●	○	○	◐	A_{00}
M. silvaticus Hagem	●	○	○	◐	A_{01}
Mycelium radicis atrovirens Melin	●	●	●	○	B
Oidiodendron maius Barron	◐	●	◐	○	A_{02}, B
O. periconioides Morall	◐	●	◐	○	A_{02}, A_2
O. tenuissimum (Peck) Hughes	◐	●	◐	○	A_{01}/A_{02}
Oidiodendron sp. 1	○	◐	◐	○	B
Paecilomyces farinosus (Dickson ex Fr.) Brown & Smith	◐	○	○	◐	A_1/A_2
Penicillium brevi-compactum Dierckx	●	●	◐	○	A_{00}
P. daleae Zaleski	●	●	●	○	A_1/A_2
P. cf. *diversum* Raper & Fennell	◐	●	●	○	A_{00}
P. frequentans Westling	◐	●	◐	○	A_{00}
P. nigricans Bain. ex Thom.	●	●	◐	○	A_{00}
P. nigricans ser.	●	○	○	○	A_{00}
P. soppii Zaleski	●	●	◐	○	A_{01}
P. spinulosum Thom I	●	●	●	○	A_{01}
Penicillium sp. 1	◐	●	◐	○	A_{00}
Penicillium sp. 2	◐	●	◐	○	A_{00}, A_{01}
Penicillium sp. 4	○	◐	○	○	A_{02}
Phialophora mutabilis grp.	●	●	○	○	B
Rhinocladiella compacta (Carrión) Schol-Schwarz	○	●	○	○	A_{02}, A_1/A_2
Septonema chaetospira (Grove) Hughes var. *pini* Bourchier	○	○	○	○	A_{01}/A_{02}
Thysanophora penicillioides (Roum.) Kendrick	○	○	○	○	A_{00}
Tolypocladium geodes W.Gams	○	○	○	◐	(B)
Trichoderma hamatum (Bon.) Bain.	●	●	●	◐	A_1/A_2
T. polysporum (Link ex Pers.) Rifai	◐	●	●	○	A_{00}, A_{01}
T. viride Pers. ex S.F. Gray	●	●	●	◐	A_{00}, A_{01}
Verticillium bulbillosum W.Gams & Malla	●	●	◐	●	A_{01}
V. lecanii (Zimm.) Viégas	●	○	○	●	A_{01}, A_{02}
Unidentified 1 (CBS 830.73)	◐	○	○	○	A_{01}

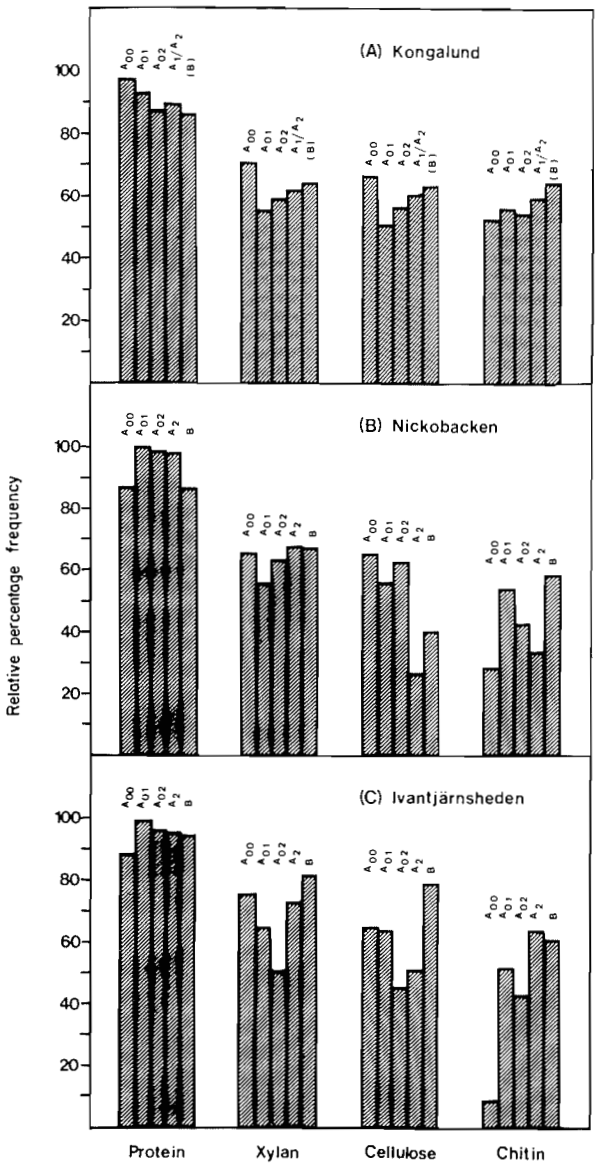

FIG. 1. The relative degrading capacity (percentage fungal isolates with any degrading potential of total number of isolates) in three different coniferous forest soils. (A) Kongalund, a planted spruce forest (*Picea abies*) in the south of Sweden. (B) Nickobacken, a mixed coniferous forest (*P. abies, Pinus sylvestris*) in central Sweden. (C) Ivantjärnsheden, a Scots pine forest (*P. sylvestris*) in central Sweden. The sites have been described elsewhere (Söderström and Bååth 1978).

taxonomic dissimilarity between two fungal communities does not necessarily result in dissimilarity in physiological capacity.

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